REMARKS/ARGUMENTS

Status

Claims 1-20 are under examination. Applicants acknowledge with appreciation the Examiner's withdrawal of the species election requirement.

Double Patenting

Applicants disagree that claims 8 and 9 are duplicates or else so close in content that they both cover the same thing, as asserted by the Office. In claim 8, the first and third cleavage sites *or* the second and fourth cleavage sites *or* the 5-prime and 3-prime cleavage sites *or* various combinations of cleavage sites can be cleaved by a Type IIS restriction enzyme. In claim 9, all of the cleavage sites are cleaved by a Type IIS restriction enzyme. Therefore, claim 8 and claim 9 differ in scope.

***Rejections Under 35 USC 102 based on Siegal* et al. *or Mandecki* et al.**

Claim 1 was rejected as anticipated by Siegal *et al.* or Mandecki *et al.* Claim 1 has been amended for clarity. It is now clarified that the three DNA molecules are three different DNA vectors, and that the selection of the ligation product is based on a selectable marker of one of the vectors. Support for the amendment is replete in the specification (see, e.g., paragraphs [0177] to [0198]).

Siegal *et al.* described a method for directional cloning of a single fragment ("insert fragment") into a single vector. The method entails restricting the vector into two fragments and ligating the two vector fragments and the one insert fragment (hence the statement by the Office that Siegal teaches a ligation reaction containing three DNA fragments) to regenerate the vector containing the insert. In the method described by Siegal, both the insert fragment and both vector fragments are gel-purified prior to ligation. In contrast to the instant invention, which enables rapid cloning of multiple DNA segments in a predetermined order to produce a long DNA segment without the need to gel-purify the ligation precursors or products, Siegal described cloning of a single gel-purified segment into a vector. Claim 1 has been amended to make clear that at least three different DNA vectors, each comprising a DNA segment, are used to result in

product that comprises at least three DNA segments in a predetermined order. Applicants respectfully request this rejection be withdrawn.

Mandecki *et al.* described a method in which a plurality of oligonucleotides are introduced into a vector by bridge mutagenesis (described in the reference). This method allows cloning of a plurality of different inserts without a ligation step (the insertion of the oligonucleotide sequence into the vector involves *in vivo* DNA repair rather than *in vitro* ligation). In this method, a single vector is used and the vector molecules have a single insert (or no insert). Cells containing vectors with an insert can then be identified by routine methods (see page 103, col. 1, first full-paragraph (section (b)). This appears to be the selection step asserted by the Office to constitute selection of the ligation product. (The Office refers to col. 2, rather than col.1 of page 103: Both describe selection of bridge-mutagenesis products.) Insofar as the selection described in Mandecki is selection for clones having a single insert, resulting from bridge mutagenesis, and not involving ligation, the Office is mistaken in asserting that Mandecki teaches selection of the product of the ligation reaction.

The vector used by Mandecki includes *Fok*I sites that flank the insertion site. According to the Mandecki method, the bridge mutagenesis clones are digested with *Fok*I to excise the inserts. Each insert is then gel-purified (see, e.g., page 103, col. 2, first partial paragraph). The gel-purified fragments are then ligated. The resulting ligation product is itself gel-purified, restriction digested, and cloned into a vector (see Figure 4 legend). Mandecki described a method wholly unlike the current invention which among other differences did not include providing at least three different DNA vectors or selecting a ligation product based on a selectable marker of one of the vectors. Accordingly this reference did not anticipate claim 1 as amended. Applicants respectfully request this rejection be withdrawn.

***Rejections Under 35 USC 102(e) based on Santi* et al.**

Claims 1-6 and 18-20 were rejected as anticipated by Santi *et al.* (US 2004/0166567).[1] The Santi *et al.* application is related to the instant application and represents a major advance in cloning technology. The Santi *et al.* application broadly describes and enables what is

[1] US 2004/0166567 and the instant application both are assigned to Kosan BioSciences, Inc.

sometimes called "ligation by selection" gene synthesis and other methods. As described in US 2004/016656, the new method was used to build a synthetic 31,656-base pair gene. Also see Kodumal et al., November 2004, *Proc. Nat. Acad. Sci.* 101:15573-78(copy enclosed).

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Moreover, it is well-established that a valid patent may be obtained even if the claimed invention is dominated by another patent.

The Office writes that in Santi "the synthons are provided in vectors (type I, II and III DNAs) that each contain a first selectable marker." However, Santi did not specifically describe a method in which three different vectors, each of which comprises a different synthon, having the characteristics of the Type I, II and III vectors recited in claim 2, are ligated together simultaneously to produce a ligation product that comprises sequences from each DNA segment in a pre-determined order. Thus, the instant claims are not anticipated.

Rejections Under 35 USC 103(a)

Claims 1-20 were rejected as allegedly unpatentable over Lebedenko *et al.* and Gokhale *et al.*, "as evidenced by" Slater *et al.* Applicants are unfamiliar with the phrase "as evidenced by" in this context and understand the rejection to be based on Lebedenko *et al.*, Gokhale *et al.*, and Slater *et al.*, taken together. The Office is respectfully requested to clarify whether the Slater *et al.* reference, which was published after the filing date of the instant application, is being relied on by the Office as prior art.

Applicants respectfully submit that the Office has failed to establish a *prima facie* case of obviousness.

Lebedenko *et al.* described synthesis of a gene encoding mature human IL-1α using a method that includes ligation of three DNA fragments (corresponding to exons 5, 6 and 7 of the IL-1α gene). The sequences encoding each of the exons were amplified using PCR and the PCR products each cloned into pUC19 vector. The vectors were digested with BamHI and/or Eco31I to excise the fragments (page 6758, col. 1). Each of the resulting four fragments (exon 7 yielded two fragments due to the presence of a *Eco*31I site) was purified by gel electrophoresis and

electroelution. The four fragments were then ligated together, the ligation mixture was treated with *Bam*HI to cleave undesired ligation products, and the desired ligation product was obtained by gel electrophoresis and electroelution. The gel-purified ligation product was then cloned into pBR540 vector. See page 6760, col. 2, first full paragraph.

Gokhale *et al.* described recombining modules from naturally occurring PKSs using conventional recombinant techniques. Gokhale *et al.* showed that modules that are not naturally found in the same polypeptide can be combined to produce catalytically functional products. This reference is wholly unrelated to the instant invention. It is cited by the Office solely to show motivation to clone PKS genes.

The Office asserts "it would have been obvious . . . to use the method of Lebedenko et al. to obtain synthetic PKSs as taught by Gokhale *et al.* with a reasonable expectation of success." [2] Applicants respectfully disagree. Lebedenko *et al.* described the cloning of a short (roughly 500-basepair) DNA by a process involving multiple cycles of electrophoresis, elution, gel filtration and *de novo* cloning. A prototypical PKS-encoding sequence (DEBS gene cluster) is over 30,000 bp in length (comprising shorter sequences encoding individual modules). One of skill using the Lebedenko *et al.* method would have had virtually no expectation of success of synthesizing a PKS gene (and therefore would not have had even a motivation to try).

The Office further states that "with respect to the DNA molecules each having a selectable marker and a counter-selectable marker, this is not innovative, the prior art teaches the use of diverse selectable and counter-selectable markers and combinations thereof (see Slayter et al.). One of skill in the art would know to use the right combination of selectable and counter-selectable markers for the selection of the desired product." Lebedenko cloned three DNA fragments into pUC19 vector, isolated the fragments using PAGE, restricted and ligated the gel-purified fragments, and isolated the ligation product by PAGE. The Office fails to suggest any reason the Lebedenko should be modified to add selectable and counter-selectable markers to the DNA fragments to be ligated or vectors carrying them. Applicants respectfully submit that it is not relevant whether use, generally, of selectable and counter-selectable markers was known. This basis for rejection is rather like saying that "DNA" was known, or "restriction enzymes"

[2] It is not entirely clear what the Office means by synthetic, and whether the Office is using this term as a synonym for "recombinant."

were known. The claimed *methods* were not known and are not suggested by the references relied on by the Office.

As to the assertion that one of skill in the art would know to use the "right combination" of selectable and counter-selectable markers for the selection of the desired product, this is wholly unsupported. The Office fails to suggest how or why the Lebedenko method would have been modified to use three DNA vectors as disclosed by the instant inventors.

The Office also states that "with respect to the limitation of the presence of at least two Type 3 DNA molecules [referring to claim 15], one of skill would know to use more than one molecule, depending on the gene needed to be synthesized." The Office has ignored the fact that, as is clearly discussed in the specification, Type 1, 2 and 3 DNA molecules each have different features and functions and are used in combination in the claimed method. The assertion by the Office that "one of skill would know" to use more than one molecule simply does not provide any legally cognizable basis for an obviousness rejection.

The references cited by the Office neither described nor suggested the claimed invention. Applicants submit that no *prima facie* case of obvious has been established. Applicants respectfully request this rejection be withdrawn.

Conclusion

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

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Respectfully submitted,

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